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DIALYSIS – TRANSPLANTATION

Nonheart-beating kidney donation: Current practice and future developments

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Nonheart-beating kidney donation: Current practice and future developments.

Background. Nonheart-beating kidney donation (NHBD) is gaining acceptance as a method of donor pool expansion. However, a number of practitioners have concerns over rates of delayed graft function, acute rejection, and long-term graft survival. The ethical issues associated with NHBD are complex and may be a further disincentive. Tailored strategies for preservation, viability prediction, and immunosuppression for kidneys from this source have the potential to maximize the number of available organs. This review article presents the current practice of NHBD kidney transplantation, examines the results and draws comparisons with cadaveric kidneys, and explores some areas of potential development.

Methods. A review of the current literature on NHBD kidney donation was performed.

Results. The renewed interest in NHBD kidneys is driven by a continuing shortfall in available organs. Those centers involved in NHBD report an increase in kidney transplants of the order of 16% to 40% and there is no evidence that the financial costs are higher with NHBDs. The majority of experience comes from Maastricht category 2 NHBDs, where an estimation of warm time is possible. This is generally limited to 40 minutes. There are variations in the technique for kidney preservation prior to retrieval, but most centers use an aortic balloon catheter. Much work has looked at the ideal technique for kidney preservation prior to implantation. Evidence suggests that machine perfusion produces the best initial function rates, decreased use of adjuvant immunotherapy and fewer haemodialysis sessions than static cold storage.

Conclusion. Despite being associated with poorer initial graft function, the long-term allograft survival of NHBD kidneys does not differ from the results of transplantation from cadaveric kidneys. Further, serum creatinine levels are generally equivalent. Constant reassessment of the ethical issues is required for donation to be increased while respecting public concerns. Use of viability assessment and tailoring of immune suppression for NHBD kidneys may allow a further increase in donation from this source.

Key words: nonheart-beating kidney donation, heart-beating donors

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Renal transplant activity is of the order of 1800 per year in the United Kindom and there were 4831 people awaiting kidney transplantation at the end of August 2001 (United Kingdom transplant data). This shortfall in kidneys is exacerbated by two trends decreasing the number of available brain stem–dead (heart-beating) donors. These are a reduction in fatal road traffic accidents and a decline in the number of deaths from intracerebral hemorrhage. On top of the decrease in available organs is a constant expansion in the number of potential recipients [1]. There is, therefore, a continuing challenge to maximize donor organ availability. One approach for reducing the discrepancy between required and available kidneys for transplantation is the use of nonheart-beating donors (NHBD). This paper presents the current practice of NHBD kidney transplantation and explores some of the areas of potential development.

DEFINITION OF NHBD KIDNEYS

The distinction between heart-beating donors (HBDs) and NHBDs lies in the criteria used to diagnose death; in HBDs brain-stem death criteria are used, whereas cardiac criteria are applied to NHBDs (Table 1) [2].

NHBD kidneys suffer a period of warm ischemic damage [the warm ischemic time (WIT)], calculated as the time between cardiopulmonary arrest and initiation of external cardiac massage with artificial ventilation. It is often of unknown duration and is a damaging period since there is no perfusion to support renal cell homeostasis. The use of NHBD is not new; prior to brain-stem death legislation, kidneys from NHBD were routinely retrieved as the only organ resource. In those countries such as Japan, where brain-death criteria are not generally accepted [3] and organ procurement from HBDs is legally prohibited without the patient's written consent before brain death [4], NHBDs are still the major source of kidneys [5]. During the late 1980s, investigators began to look again at safe methods of renal preservation for NHBDs with the aim of increasing the donor pool to

Table 1. Brainstem and cardiac criteria for diagnosis of death

Criteria for brainstem death (HBD)
- The underlying pathologic lesion should be understood;
- There should be no pharmacologic, metabolic, or hormonal influence;
- Pupillary, corneal, occulocephalic, vestibulo-occular, and gag reflexes should be absent;
- No pain response to stimulation in the distribution of the fifth cranial nerve; and
- A rebreathing test with 100% oxygen should be delivered to maintain satisfactory oxygenation, while ventilation is switched off. The rise in arterial pCO_2 should not stimulate respiration.

These tests are performed by two experienced clinicians on two separate occasions.

Criteria for cardiac death

The criteria for brain-stem death need not be fulfilled for nonheart-beating donation (NHBD) candidates. When cardiac arrest has occurred and the medical team has decided that further treatment is pointless, then the diagnosis of death is simple. The criteria are:
- Deep coma
- Absence of pulse
- ECG evidence of asystole

Cardiac death in the context of potential organ donation is defined as occurring after 30 minutes of unsuccessful cardiopulmonary resuscitation (CPR) under hospital conditions. Resuscitation must include external cardiac massage, intubation, ventilation, defibrillation (if indicated), and appropriate intravenous medication. "Unsuccessful" means that these measures did not achieve spontaneous contractile cardiac activity or peripheral circulation.

meet demand. A number of centers are now involved in NHBD transplantation but many transplant physicians are reluctant to use such kidneys; the concerns include high rates of delayed graft function, poor renal function, increased expense secondary to greater medication, dialysis use, longer hospital stay, and alleged inferior graft survival rates compared to HBD. The extent of these concerns was highlighted in a questionnaire on the need to take specific consent from recipients of NHBDs; half of the transplant surgeons questioned thought that a NHBD kidney increased the risk of transplantation, 29% thought it did not, and 21% were unsure [6].

There is an important distinction between controlled and uncontrolled NHBD. Controlled NHBD are those in whom cardiac arrest is awaited by the transplant team; ischemic time is kept short and other organs may be harvested along with the kidney. Uncontrolled NHBD are those donors who suffer cardiac arrest suddenly before scheduled organ harvesting can be arranged [7]; hence, the WITs are longer. At the First International Workshop on NHBD in Maastricht, four categories of NHBD were described [8], both to aid legal and ethical discussion and to highlight possible differences in viability. Category 1 includes the victims of accident and suicide (some centers exclude suicide victims from their programs) who are found dead at the scene and resuscitation is deemed pointless (e.g., fatal cervical spine fracture). Problems with using this group as donors are unknown WIT and difficulty in contacting relatives quickly enough for consent. There is little experience of using category 1 donors reported in the literature. Category 2 donors are the mainstay of the NHBD pool in Europe and Japan, and are mostly victims of sudden cardiac (the majority) or cerebral catastrophe who are brought to emergency departments while being resuscitated by ambulance personnel or who die in the department. An estimation of WIT is easier because the personnel are able to provide information on the time of collapse, although there is considerable variation in WIT because of the heterogeneity of the group. Other sources include patients suffering isolated brain injury, anoxia and stroke, and victims of major trauma who die soon after hospital admission [9]. Category 3 encompasses patients who are dying, often on an intensive care unit. These were the traditional donors prior to introduction of brain-death legislation, and still account for a greater proportion of donors in the United States than in other countries. The family will have agreed to organ donation, and after medical support is withdrawn, cardiac arrest is awaited. A double-balloon triple-lumen (DBTL) perfusion catheter (see later discussion) is introduced 2 to 10 minutes after the patient is declared dead by cardiac criteria. The main ethical issue in this category is that the decision to donate occurs before the patient is dead.

Category 4 comprises patients who suffer unexpected cardiac arrest during or after determination of brain death.

NHBD contribution to the donor pool

The most encouraging figure for the contribution of NHBD kidneys to transplant programs has come from Daemen et al [10], who reported 40% of their kidneys were accounted for by NHBDs. However, due to faster decreases in the supply from other sources, this 40% contribution did not increase the overall transplant activity. For the Leicester group, Varty et al [11] reported 38% of donors were NHBD for the first year of use of this resource, and later Nicholson [12] reported that NHBD accounted for 21% of total transplants. Increased transplant activity of 20% [13] and 16% [8] have also been reported. Light et al [14] stated that the number of NHBD opportunities equals that of HBD, but others have suggested that there are twice as many potential NHBDs as there are HBDs [15]. Terasaki, Cho, and Checka [16] claim that if all potential NHBD kidneys were retrieved, waiting lists for kidney transplants would be eliminated. Further, they suggested that there would

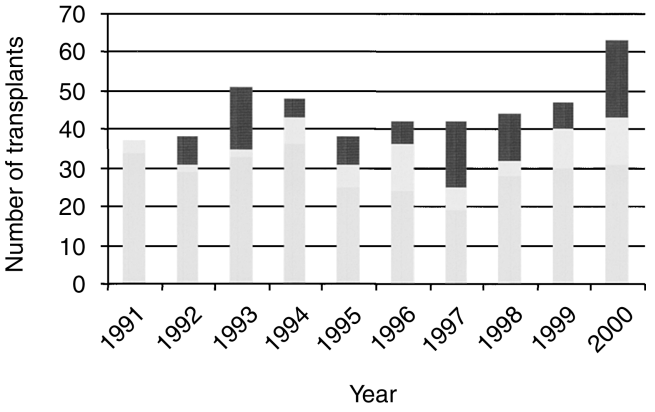

Fig. 1. The number of renal transplants performed in a 10-year period in Leicester, by donor source [18]. Symbols are: (■), live donors; (□), nonheart-beating donors (NHBD); (■), heart-beating donors.

be no need to use living donors, thereby avoiding exposure of otherwise healthy individuals to potential morbidity. In a retrospective review of 603 in-patient hospital deaths, Daemen et al [17] found that utilization of this resource would have yielded up to 56 NHBDs, increasing transplant activity 4.5-fold.

Workload and Costs generated by a NHBD program

Figure 1 shows the contribution by donor type for the Leicester transplant program. The figures for 1992 to 2000 show a total of 350 kidney transplants performed; 77 (22%) kidneys came from NHBDs, 224 (64%) from HBDs, and 49 (14%) were from live donors [18]. In the 3-year period, 1992 to 1995, the authors' unit had 73 NHBD referrals (146 potential kidneys), with 38 kidneys (26%) retrieved [19]. This is a sizable workload, with considerable costs implications.

Maintaining a patient on hospital dialysis per year costs £25,000 to £31,000, with home dialysis estimated at £16,000 to £25,000, depending on region. The cost of a renal transplant procedure is approximately £14,500, and the annual maintenance cost posttransplant is £6500 (1998 figures, Department of Health NHS Renal Purchasing Guidelines data), an obvious considerable annual saving over dialysis. Thus, the more patients than can be converted from dialysis to transplantation, the greater the cost savings. For immunosuppression, the costs are equal for HBDs and NHBDs, as similar regimens are used regardless of donor source. The cost rises significantly if acute rejection episodes are treated with immunoglobulins, but the evidence (see later discussion) suggests there is no difference in acute rejection rates between HBDs and NHBDs.

METHODS AND TECHNICAL ASPECTS: THE LEICESTER APPROACH

All patients who have sustained, or are likely to sustain, permanent circulatory arrest are considered as potential NHBDs. A checklist (Table 2) is used by the responsible physician (usually an emergency department registrar or consultant) to ensure that the donor is eligible. If there are no exclusion criteria, the transplant coordinator is notified and the transplant team attends. The team consists of the transplant coordinator, consultant, and registrar. The donor is left for a 10-minute period from the time of pronouncement of cardiac death to ensure brain death. This length of time has been a topic for debate (see later discussion). After this period, the donor is placed on the mechanical external compression and artificial ventilation machine [20], a device originally developed to assist with cardiopulmonary resuscitation (CPR). It provides external cardiac compressions at a set rate and depth, along with artificial ventilation with 100% oxygen via an endotracheal tube, with the aim of limiting ischemic damage to the kidneys. No further action is taken until consent for placement of femoral artery and vein catheters is given by relatives. Once consent is obtained (75% to 80% of potential donors [10, 20]) a right groin crease incision is made and the common femoral artery is isolated and controlled. Through a transverse arteriotomy,

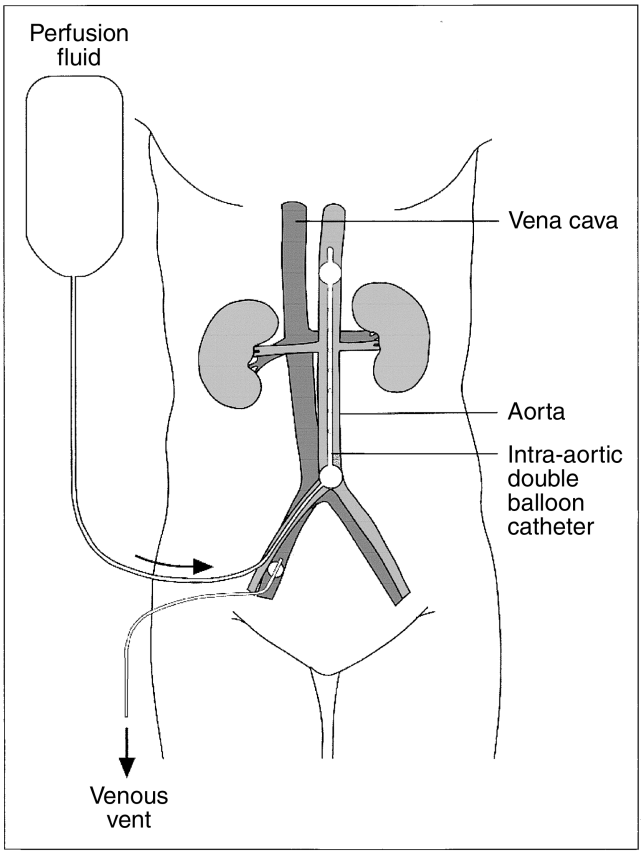

Fig. 2. The in situ perfusion technique showing correct positioning of the double-balloon triple-lumen (DBTL) catheter in the aorta, inflated balloons and perfusion fluid attached to the catheter. The femoral vein is vented.

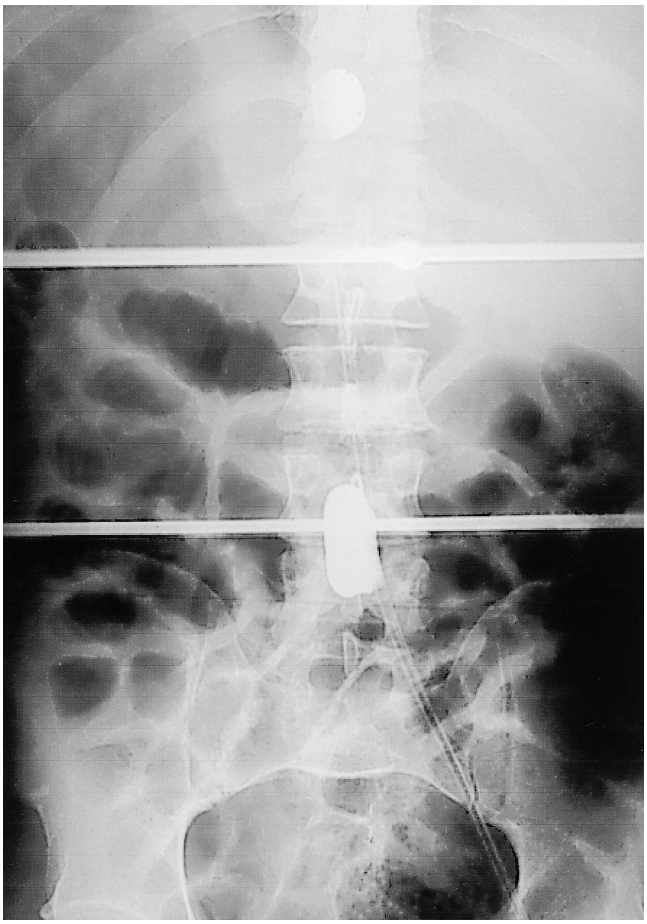

Fig. 3. A check radiograph showing correct positioning of the double-balloon triple-lumen (DBTL) catheter. The balloons are filled with dilute contrast.

the DBTL catheter is inserted into the aorta [21]. The caudal-most (abdominal) balloon is inflated with dilute contrast, and the catheter is pulled back until it lodges at the aortic bifurcation. The cranial-most (thoracic) balloon is then inflated with contrast and 150 mL blood is withdrawn for virology, syphilis serology, blood grouping, and tissue typing. The catheter is then connected to the cold (4°C) perfusion fluid reservoir, and in situ perfusion is started with hyperosmolar citrate solution. Heparin and phentolamine are administered in the first bag of preservation fluid. Heparin is thought to reduce intravascular thrombosis, and phentolamine may reduce intrarenal vasospasm. The femoral vein is controlled, and a 14 Ch urinary catheter is inserted to vent the circulation. A typical volume of perfusion fluid used is 10 to 15 L. Assessment of adequacy of in situ perfusion is difficult; during perfusion, the flanks can be palpated to assess temperature and there is indirect evidence of kidney perfusion if the flanks are cold. Further evidence of correct placement is gained if the venous outflow clears as it becomes diluted with perfusion fluid. A check radiograph of balloon position is taken to confirm correct placement

Table 2. The exclusion criteria for NHBDs [19, 20]

1. Cardiac and circulatory arrest does not last longer than 40 minutes (varies between centers). This is the period of absolute circulatory arrest and does not include periods of resuscitation.
2. The patient is between 16 and 60 years old.
3. The patient does not belong to a high-risk group for human immunodeficiency virus (HIV), or hepatitis B or C infection. There should be no signs of intravenous drug abuse.
4. The patient has no history of primary kidney disease, uncontrolled hypertension, or complicated insulin-induced diabetes mellitus (IDDM). There are no signs of intravascular coagulation with anuria and no signs of malignancy other than a primary (nonmetastatic) cerebral tumor.
5. There are no signs of sepsis or serious infection.
6. Patients who have died after assisted suicide or euthanasia are excluded from some protocols.

(Figs. 2 and 3). Again, this gives indirect evidence of perfusion and, if the catheter is incorrectly placed, it can be repositioned [22]. A misplaced catheter most commonly occurs if the caudal balloon is pulled back too far; in this circumstance the contralateral leg will be perfused and feel cold. During the procedure, the transplant coordinator will reapproach the family members and ask for consent for the donation of kidneys, corneas, bones, and heart valves. Prior to transfer to theater for donor nephrectomy, the family members are offered the opportunity to see the body, and the coroner and coroner's pathologist are consulted. We attempt to complete the donor nephrectomy within 2 hours of commencement of in situ perfusion. Further in-depth description of the technique is given by Heineman, Daemen, and Kootstra [23].

WIT and time limits

The length of the WIT correlates well with graft damage and there is a point at which organs become nonviable. Although there is no strict maximum WIT or in situ perfusion time, some guidelines have been suggested, and these reflect estimates of the amount of reversible damage the kidney can tolerate. Limits of 30 minutes [24], 35 minutes [25], and 45 minutes [26] have been advocated. Functional recovery in animal models has been achieved after much greater WITs (e.g., 120 minutes [24, 27] and 140 minutes [28, 29]). In practice, allowable maximum WITs vary in a qualitative manner, for example, a young and previously fit donor may be allowed a longer WIT than an older donor.

Low yield of NHBD kidneys

There is a low yield of useable kidneys from NHBDs. Over a 3-year period at the authors' unit there were 73 referrals, giving 146 potential kidneys. The retrieval rate was 24 of 73 (33%). The reasons for this low rate were analyzed, and included refused consent ($N = 13$), relatives unavailable for consent ($N = 4$), technical problems ($N = 10$), long asystolic period ($N = 8$), medically unsuitable donor ($N = 8$), and unavailability of transplant staff ($N = 1$).

Table 3. A comparison of rates of PNF, DGF, and acute rejection at five centers performing non-heart beating kidney (NHBD) transplantation

	Nicholson [38]	Ohshima [39]	Tanabe [40]	Cho [15]	Wijnen [35]
Primary nonfunction (PNF)	9%	4%	9%	4%	14%
Delayed graft function (DGF)	84%	70%	78%	48%	60%
Acute rejection	29%	45%	51%	19%	48%

Comparisons should be made with caution due to variations in donor category, perfusion techniques, human leukocyte antigen (HLA) matching and immunosuppression protocols.

Of a potential 48 kidneys, 44 were obtained and 38 of these suitable 44 kidneys were transplanted, giving a ratio of actual transplants to total number of kidneys theoretically available as 38 of 146 (26%) [19].

Do NHBD kidneys function as well as HBD kidneys?

Ischemic damage to NHBD kidneys. Central to the debate over the safety and efficacy of NHBD kidney use is whether warm ischemic damage causes unacceptable levels of irreversible kidney damage. Consideration must be given to the effect of warm ischemic damage on rates of primary nonfunction (PNF), delayed graft function (DGF), acute and chronic rejection, patient and allograft survival, renal function, and quality of life.

When considering the results of NHBD kidneys, it is important to differentiate between different categories, as the warm times can differ greatly. In a retrospective study of category 3 and 4 donors (in which the preagonal phase may be prolonged), Rowinski et al [30] concluded that events around the time of brain-stem death such as profound metabolic, hemodynamic, and hormonal changes may play a more important role in the pathogenesis of renal damage than warm ischemia. Alvarez et al [31] showed that kidneys from ITU-based NHBD had poorer short- and long-term function, and were associated with a greater rate of PNF than NHBD kidneys procured from the emergency department, and this was strongly associated with periods of hypotension prior to retrieval. Moreover, White et al [32] describe a series of transplants in which, despite higher levels of DGF (93% NHBD and 17% of cadaveric), graft survival at 3 years was significantly better for NHBDs than HBDs (84% vs. 73%). One explanation for this is that the NHBD kidneys were not subjected to the harmful events associated with brain-stem death. A pathophysiologic explanation for this phenomenon was proposed by Takada et al [33], where the up-regulation of genes for proinflammatory mediators was demonstrated in an animal model of brain-stem death. It is postulated that if these results are relevant to humans, then organs from HBDs will be more prone to early host inflammatory and immune responses, as NHBDs suffer sudden circulatory arrest, which allows no time for gene up-regulation.

Rates of PNF. The rate of PNF of NHBD kidneys ranges from 8% to15% [11, 34, 35], which is higher than the 2% to 5% quoted for HBD kidneys [36, 37] (Table 3). Shiroki et al [41] claimed that the PNF rate is highest in those NHBD kidneys with a history of WIT longer than 30 minutes. Tanabe et al [40] disagreed, stating that length of WIT had no relationship to the rates of PNF, but no WIT was longer than 30 minutes in their study. In HBDs, PNF is most often due to the vascular complications of arterial or venous thrombosis, and one study has shown no significant difference in the rates of vascular thrombosis (1%) when comparing NHBD and HBD [38]. The potential role of viability testing prior to implantation is discussed later.

Rates of DGF. Almost all authors find the rate of DGF higher with NHBD kidneys. DGF occurs in 20% to 60% of HBD kidney transplants [14, 31, 32, 35] and 50% to 100% of NHBD transplants [36, 40] (Table 3). This higher rate of DGF in NHBD kidneys is likely to be a consequence of marked acute tubular necrosis secondary to the long WIT. Not all DGF is due to acute tubular necrosis, although acute tubular necrosis is the most common cause and it is, therefore, unsurprising that kidneys harvested from NHBDs have a higher incidence of acute tubular necrosis than those from a HBD [34]. It is important to note that WIT is not the only variable determining the likelihood of DGF; Yokoyama et al [42] found that long duration of pretransplant recipient dialysis and increased body weight were also correlated with posttransplant early graft dysfunction.

Schlumpf et al [43] reported similar results for NHBD and HBD for rates of acute tubular necrosis and 1-year graft survival if WIT was kept to less than 20 minutes. These donors, however, had a lower mean age (35 years) than other groups, which may have skewed their findings. Another point, previously mentioned, is that the insults suffered by HBD kidneys (the profound metabolic and hormonal changes associated with brain-stem shock) may be as significant in the long-term as the warm ischemic damage suffered by NHBD kidneys.

Rates of acute rejection. During and after recovery from DGF, it has been postulated that kidneys suffer more frequently from acute rejection; the proposed mechanism is up-regulation of human leukocyte antigen (HLA) class II antigens during DGF. The literature suggests this is true for HBD, but it does not seem to hold true for NHBDs [35, 44, 45]. Equivalent acute rejection figures for NHBDs and HBDs have been reported by many authors [34, 46–48]. Only one large comparative

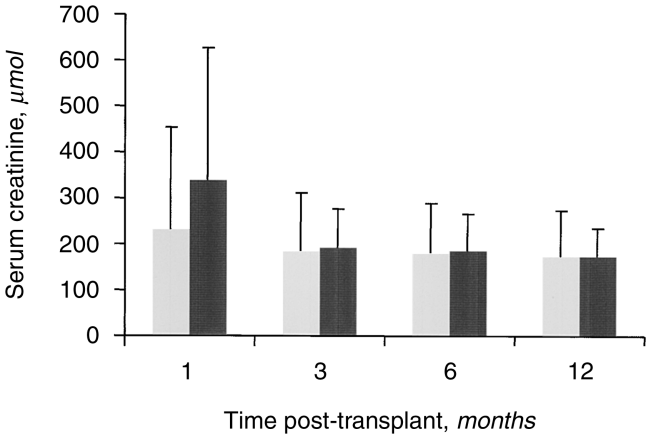

Fig. 4. Posttransplant serum creatinine by donor source [35]. Symbols are: (▩), heart-beating donors (HBD); (■), nonheart-beating donors (NHBD). Serum creatinine is presented as mean ± standard deviation of the mean.

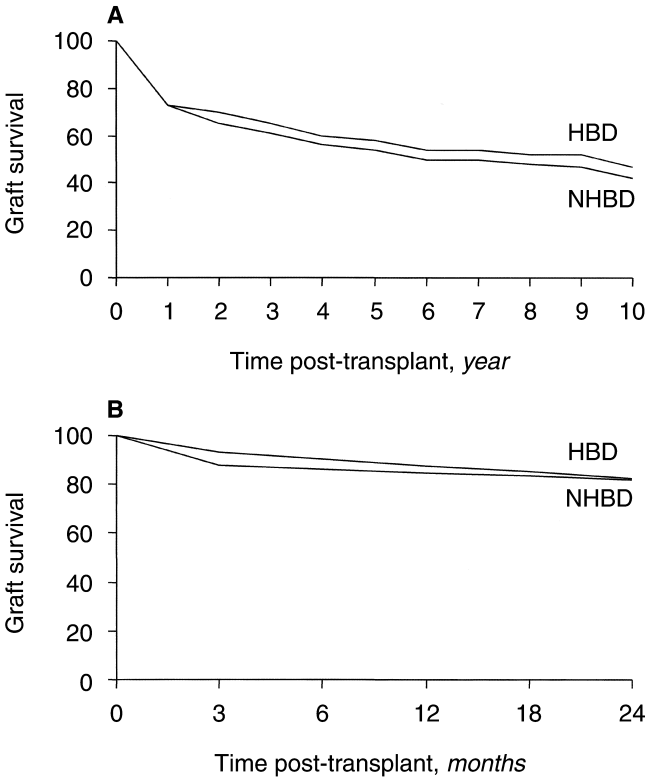

Fig. 5. (*A*) Representation of renal allograft survival from heart-beating (HBD) and nonheart beating (NHBD) donors from Eurotransplant data [35]. (*B*) Representation of renal allograft survival from heart-beating (HBD) and nonheart beating (NHBD) donors from the Kidney Transplant Registry of the United Network for Organ Sharing [15].

study [15] shows higher acute rejection rates for NHBDs (19%) compared to HBDs (14%), but it is not clear if the statistical significance has clinical relevance.

Rates of chronic rejection. There is a paucity of data on chronic rejection rates in NHBDs. The process of chronic rejection is poorly understood, but seems multifactorial with a final common pathway of a stereotyped response of the kidney to early injury. Thus, the warm ischemic damage suffered by NHBD may theoretically augment chronic rejection, either in pace or frequency. The only comparative study of HBDs and NHBDs, with a follow-up period of 95 months, showed no difference in chronic rejection rates [36].

Renal function. There is disagreement in the literature over how effectively NHBD kidneys function. Some studies show they can achieve early serum creatinine levels in the normal range [11, 15, 37, 49, 50], while others illustrate poorer graft function in both the short- and long-term [51, 52]. Castelao et al [34] found that for up to 1 year, serum creatinine is significantly higher in NHBDs than HBDs, but from then, until 6 years (the end of the study), the difference disappears. Others have confirmed this; in the medium term, NHBD kidneys achieve a good level of renal function with a mean serum creatinine at 12 months of 174 μmol/L [53] and a median of 199 μmol/L at 18 months [11]. These figures are comparable to HBD kidneys. Other investigators have shown significantly higher serum creatinine for NHBDs compared to HBDs. In one report [18], NHBD kidneys gave consistently higher creatinine levels (on the order of 40 to 50 μmol/L) over 5 years compared to HBD kidneys. However, these levels were stable and not rising. The implication is that NHBD kidneys have a reduced functioning glomerular mass due to initial ischemic damage, but once transplanted there is no evidence of further deterioration up to 5 years.

On balance, the literature seems to suggest that during the first month posttransplant, serum creatinine is relatively high in NHBD kidneys, but this improves with time as the renal tubular epithelium is regenerated (Fig. 4).

Renal allograft survival. Despite the poorer graft function of NHBD kidneys in the early posttransplant period, the majority of data show no significant differences in allograft survival between NHBD and HBD kidneys at 3 years [15], 5 years [15, 40], and 10 years after transplantation [35] (Fig. 5). An in-depth review of 24 years experience of NHBD and HBD kidneys again revealed no difference in graft survival [54]. The influence of WIT on NHBD kidney graft survival was investigated by Morpurgo, Rigotti, and Ancona [27], who found a decrease in graft survival with increasing ischemic time. Others have found that length of ischemic time does not influence rates of graft loss [40, 52, 55]. It would seem, then, that long ischemic time causes reversible graft problems at an early stage, in terms of PNF and DGF. Later, NHBD grafts appear to perform as well as those from HBDs. NHBD kidneys meet The British Transplantation Society guidelines [56] for allograft survival at 1 and 5 years of 80% and 60%, respectively. This has obvious relevance to NHBD programs and is evidence

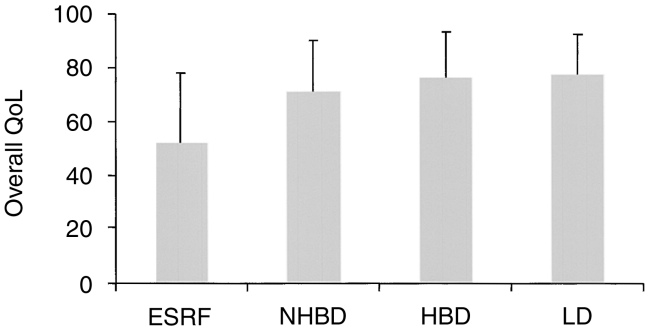

Fig. 6. Quality-of-life scores for end-stage renal failure (ESRF) and transplant recipients according to source, using K_D-QOL SF36 scoring system. Abbreviations are: NHBD, nonheart-beating donors; HBD, heart-beating donors; LD, live donors [18].

that the widespread reluctance to accept NHBD kidneys as a valid source of donor kidneys may be misplaced.

Patient survival and quality of life. Published data show no difference in patient survival in NHBD and HBD recipients [35, 40]. Further, there seems to be no relationship between WIT and patient survival when the NHBD kidneys are analyzed as a group [27]. The overall quality of life for recipients of NHBD organs is not significantly different than that of recipients of HBD organs or living-donor organs using the K_D-QOL scoring system (Fig. 6) [18].

One of the difficulties in comparing graft function, and graft and patient survival in NHBD and HBD are intergroup variations in WIT, causes of death, donor age, HLA matching, in situ perfusion times, method of preservation, and immune suppression protocols used. The variations make comparisons difficult and stress the need for studies of paired kidneys where possible.

POTENTIAL FUTURE DEVELOPMENTS

Results from all modes of kidney transplantation are improving due to greater understanding of the mechanisms of ischemia/reperfusion injury and warm ischemic damage, improved preservation techniques, development of viability testing prior to implantation, and advances in immune suppression. NHBD outcomes are encouraging, and areas of research aimed at limiting or partially reversing cold and warm ischemic damage, and limiting immune and nonimmune nephrotoxicity hold out hope for further expansion of the NHBD pool.

Preservation fluids

The importance of optimal preservation is highlighted for NHBD kidneys as these grafts are subject to the dual damaging processes of warm and cold ischemia. In an elegant study on the effect of warm ischemia and cold storage on renal vasculature, Hansen et al [57] showed that neither warm ischemia insult alone nor cold storage alone conferred any significant effect on the ability of renal vasculature to relax after a constricting stimulus. However, the two combined insults resulted in loss of endothelial cell function. There are clinical data to support this idea. First, cold ischemia is more damaging when superimposed on a period of warm ischamia, in terms of reduction in subsequent renal function [58]. Second, when comparing kidneys with WIT less than 45 minutes plus a cold ischemic time of less than 22 hours or longer than 22 hours, the 5-year survival rate is significantly worse for those with the longer cold time [59].

The preservation fluids used to limit ischemic damage include EuroCollins (EC), University of Wisconsin (UW) histidine-trytophan-ketoglutarate (HTK) [60], and celsior solution [61, 62], discussed below.

EuroCollins solution (EC) and University of Wisconsin solution (UW). A randomized, controlled trial of UW versus EC for NHBD kidneys showed no difference in delayed graft function rates between the groups [63], despite other data that show that UW is superior to EC in reducing the incidence of DGF, improving graft function, and extending graft survival [64, 65]. UW is more expensive than EC, but in the longer term, the costs are more than recovered because of improved kidney function [66]. Presently UW is considered the gold-standard preservative [61].

Histidine-tryptophan-ketoglutarate (HTK). HTK has been shown to reduce the incidence of DGF [67, 68] and its use results in higher rates of initial graft function and graft survival than kidneys stored in EC solution [69]. However, experimental data suggest that UW is superior to HTK in the preservation of ischemically damaged kidneys [70].

Celsior solution. This has a formulation similar to extracellular fluid, with mannitol and lactobionate added as impermeants. It has equal efficacy to UW in the context of NHBD kidneys [62], but its clinical use is currently limited to cardiac preservation [61].

Machine preservation or static cold storage?

Preservation methods aim to limit the damage inflicted by cold and warm ischemia, but as yet the optimum method has not been determined. The two approaches to preservation prior to transplant are cold storage and machine perfusion. There is a significant body of research comparing these two techniques, but debate remains. Simplicity, lower cost and ease of transport make cold storage the method of choice for the majority of renal transplant centers, and a number of studies have shown no advantage of machine perfusion over cold storage [71–74]. There is evidence suggesting machine perfusion of kidneys gives superior preservation in terms of outcome measures (principally delayed graft function) compared to cold storage. The data for this come mainly from HBD kidneys [75–78]. The beneficial effect may

be mediated by the maintenance of near-physiologic conditions. Machine perfusion is said to supply or regenerate metabolic substrates lost during warm ischemia [64], such as adenine nucleotides and glutathione [79, 80]. It maintains intracellular pH and discharges waste, dilutes or neutralizes catabolic substances [81], and decreases sodium-dependent tissue edema [82]. The improved histologic integrity may be related to improved perfusion of the renal cortex microcirculation with clearing of red cells and catabolic products of ischemic metabolism [80]. These metabolic and physiologic benefits generate a reduction in posttransplant dialysis by lowering the incidence of posttransplant acute tubular necrosis, with shorter hospital stay and increased long-term allograft survival. These benefits have been variously shown in canine autotransplantation [83], an isolated perfused kidney model [64], and in human renal transplantation [84–86]. Light et al [81] reported 95% immediate function with mechanical perfusion for HBD kidneys compared to the national figure of 75% for nonperfused kidneys [87], while Mendez et al [88] showed 65% of mechanically perfused kidneys had immediate function compared to 34% of those treated with cold storage. There is a significant rise in the rate of acute tubular necrosis if cold storage exceeds 24 to 30 hours, an effect not seen in pulsatile-perfused kidneys [88]. Southard and Belzer [89] reported only 18% acute tubular necrosis for mechanically perfused NHBD kidneys compared to the reported rates of 70% to 100% for cold storage NHBD kidneys. In a randomized comparison of paired HBD kidneys, Alijani et al [78] found that dialysis requirements posttransplantation were 63% for cold storage grafts and 17% for mechanical perfusion grafts. The power of this study lies in the exact matching of donor criteria and explantation conditions. Despite this promising evidence, a number of prospective and retrospective studies comparing mechanical perfusion with cold storage for HBD kidneys have not shown a benefit. No difference was found when comparing serum creatinine [72], dialysis requirement [72, 73, 90, 91], graft survival [72, 73, 88], or patient survival [72, 88].

Daemen, de Vries, and Oomen [92] found DGF and PNF rates for machine-perfused NHBD kidneys were higher than those of the cold-stored HBD kidneys, suggesting that mechanical perfusion cannot eliminate the inherent quality discrepancy between the two sources. However, there was a lower rate of DGF in perfused-NHBD kidneys than that reported elsewhere for cold-stored NHBD kidneys [36, 40]. Matsuno et al [28] used paired kidneys from 13 controlled NHBDs and randomly allocated them to cold storage or mechanical perfusion. Immediate function was 35% in the mechanical perfusion group compared to 8% in the cold storage group, with 1-month graft survival of 100% for mechanical perfusion and 77% for cold storage. The strength of this study lies in the comparison of paired kidneys, but the numbers were small and different solutions were used for each group.

Table 4. The advantages and disadvantages of machine perfusion (MP) compared to cold storage (CS) prior to kidney transplantation [87]

Advantages	Disadvantages
Lower incidence of DGF	Higher cost in the short term[a]
Continuous monitoring of parameters during perfusion	Endothelial injury is possible
Decreased intrarenal vasospasm	Possibility of graft damage[b]
Ability to provide metabolic support during perfusion	Logistically more complex
Potential for pharmacological manipulation	Possible equipment failure

[a] In the longer term, mechanical perfusion is thought to be cost effective in that it reduces the financial burden incurred by additional dialysis [83]

[b] Over-perfusion can cause cellular swelling [135] which may lead to graft damage. Thus a pressure or time limit may need to be applied. Further, manipulation of the renal artery for cannulation can cause endothelial damage

In summary, mechanical perfusion appears to be beneficial only for NHBD kidneys and may not confer an advantage for HBD kidneys [35, 93, 94]. Overall, it has been suggested that mechanical perfusion reduces the cost of NHBD programs because of better initial function, shorter length of stay, decreased use of antibody preparations, and fewer hemodialysis sessions [82, 83, 87] (Table 4).

Viability tests

One of the difficulties encountered in NHBD programs is judging whether an organ is fit to transplant. Successful expansion of the NHBD pool will in part be reliant on accurate predictors of which kidneys should be transplanted and which should not. The NHBD kidneys that never develop function have sustained irreversible ischemic damage before transplantation, and there are currently no reliable pretransplantation tests of viability. A pretransplantation viability test needs to be simple, quick to perform, and have a high predictive value. The test should measure the potential for recovery of the organ, rather than the amount of injury sustained.

Donor age, donor serum creatinine, and WIT. Of the donor history parameters, WIT is the only one that can discriminate between grafts that will function and those that will not [95].

Macroscopic appearance at harvesting. Bell et al [51] reported that one fourth of NHBD kidneys were rejected on the basis of visual inspection; this requires a good deal of experience and the criteria are qualitative.

Nucleotide measurements. Anoxia uncouples the process of cellular oxidative phosphorylation, resulting in a decrease in the levels of adenine nucleotides. Nucleotide levels do decrease with warm and cold ischemia, but studies so far have shown that the levels are no use in predicting later outcome [96, 97].

Perfusate lactate dehydrogenase Perfusate lactate dehydrogenase (LDH) [98] levels have been shown to be directly related to degree of ischemia [99] and are an indictor of preservation damage [100]. The levels are easily assayed, but LDH is relatively nonspecific, therefore probably not useful for viability assessment [98].

Alpha glutathione S-transferase. Alpha glutathione S-transferase (α-GST), a proximal tubular enzyme, and π-GST, a distal tubular enzyme, have been assessed as viability indicators [101]. α-GST may be a marker of damage, because it is released from the hypoxia-sensitive proximal tubular cell and levels correlate with WIT. There is no such correlation for π-GST. Total GST, as a predictor of functional recovery, was examined by Balupuri et al [102]. After introduction of machine perfusion and total GST measurement, they increased their success rate (% dialysis-free at 3 years) for NHBD from 45.5% to 92.3% by not transplanting kidneys that yielded high levels of GST.

Pressure, flow and resistance during machine preservation. These parameters, measured during machine preservation, are promising viability assessors, and the approach affords a number of advantages. First, the kidney may be resuscitated with machine perfusion. Second, perfusate chemistry can be synchronously measured. Third, there is potential for pharmacologic manipulation of the organ while it is on the machine [101]. Ischemic injury causes the release of vasoconstrictors from endothelium; together with accumulation of erythrocytes and microthrombosis, the result is diminished flow and increased resistance [101]. Some authors have shown that low intrarenal resistance and high flow alone are evidence of viable kidneys [25, 28]. Matsuno et al [103] found that perfusate flow was a reliable indicator of viability based on early functional recovery of the kidney graft; the higher the flow, the greater the level of immediate function. Tesi et al [104] rejected HBD kidneys if flow was less than 70 mL/min or intrarenal resistance was greater than 40 mL/min/100 g. With these criteria, the posttransplant acute tubular necrosis rate was only 8.6%. Kozaki et al [105] stated the criteria for accepting a kidney on the basis of vascular parameters were (*1*) a minimum flow rate of 40 mL/min/100 g at 50 mm Hg pressure, and (*2*) an increasing perfusion volume and decreasing or plateauing pressure during perfusion, while Balupuri et al [102] suggested accepting only those kidneys with a flow rate of no less than 50mL/min/100 g at a perfusion pressure of 60 mm Hg. Their results were so encouraging, they suggested that not using machine perfusion for NHBD was difficult to justify. Even kidneys with long WITs (up to 140 minutes) that have an acceptable flow on machine perfusion can function well when transplanted [28, 29]. This stresses the importance of the kidneys' behavior on the machine as a viability predictor; such kidneys would have otherwise been discarded due to the long WITs. Polyak et al [101] used papaverine, prostaglandin E_1, trifluoperazine, and verapamil during perfusion in an attempt to manipulate kidneys with low flow and high resistance. Unresponsiveness to treatment may indicate a poor-quality kidney. The weakness of all of these studies is that there is no evidence that kidneys discarded because of poor pump parameters alone would fail to work adequately.

Viability scoring based on vascular parameters, oxidative metabolism, and vascular condition demonstrates efficacy in predicting severity of acute tubular necrosis and the occurrence of PNF [106]. It offers a sensitive assay for prospective organ testing based on multiple values.

Proton magnetic resonance spectroscopy. The release of phosphorus atoms occurs with the intracellular degradation of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and adenosine monophosphate (AMP). There is a strong correlation between warm and cold ischemic damage (assessed by electron microscopy) and the phosphorous monoester (PME) to inorganic phosphorous (Pi) ratio (PME/Pi) measured by magnetic resonance spectroscopy (MRS). Viability is associated with high intracellular levels of PME and low levels of Pi [107]. The technique is noninvasive, nondestructive, sterile, rapid, and because it indirectly measures the metabolic competence of cells, it shows promise as a viability assessor [108]. The technique can also be applied to biofluids [109].

Most of these techniques of viability assessment have not yet proved to be reliable indicators of functional outcome. Additionally, many of the data are from HBD kidneys and the relevance to NHBD kidneys is a theoretical extrapolation. One of the difficulties of viability assessment is that the cold conditions of the kidney render metabolic studies difficult to interpret. If oxidative metabolism is restarted by perfusion in a more physiologic setting, then parameters may be more meaningful. The reinstatement of energy-dependent processes requires metabolic substrates and an efficient oxygen carrier in the perfusate to prevent anaerobic metabolism; an area of research to this end is the use of tissue culture–like fluid with an added perflurocarbon. The tissue culture fluid contains more than 70 ingredients, including amino acids, lipids, carbohydrates, proteins, trophic factors, vasodilators, and adenine compound substrates, adjusted to normal pH. The perflurocarbon [110] displays a linear oxygen dissociation relationship. Using this perfusate at 30°C to 32°C, Stubenitsky et al [111] showed lower oxygen consumption, glucose consumption, urine flow, and glomerular filtration rate (GFR), with increasing warm ischemic time in ex vivo kidneys. There was a strong correlation between the latter two parameters and histologic grading of acute tubular necrosis, suggesting warm perfusion may be a valuable predictive tool. These encouraging results may be due to restoration of renal metabolism, presumably a safe procedure if the correct

environment is applied. For the present time, it remains a fact that the only accurate way to predict graft function is to transplant the organ.

Immune suppression for NHBD kidneys

A number of immunosuppressive strategies have been employed for NHBD kidney recipients. Triple therapy with cyclosporine A (CsA), prednisolone, and azathioprine, or quadruple therapy with the addition of antilymphocyte globulin (ALG) have been the most commonly used protocols [3, 30, 55, 94, 112]. Nonrandomized studies of controlled NHBD have compared acute rejection and graft survival rates in groups treated with tacrolimus or CsA [113, 114]. The tacrolimus group had lower rates of acute rejection and DGF, but there was no difference in long-term graft survival. As yet there is no clear advantage of one calcineurin inhibitor over another, and further data are required. The dose-dependent acute and chronic nephrotoxic effects of calcineurin inhibitors are well described [115, 116]. Apart from chronic allograft nephropathy (CAN), one of the main problems of calcineurin inhibitors is that toxicity masks acute rejection episodes. Some centers avoid the immediate posttransplant exposure to calcineurin inhibitors by using antibody induction therapy (ALG or OKT3), adding a calcineurin inhibitor once the serum creatinine has fallen. [2, 3, 35]. Schlumpf et al [43] reported very good 1-year graft survival and function using ALG rather than early CsA in controlled NHBD. Kinukawa et al [3] performed a nonrandomized study of high-dose CsA plus prednisolone versus low-dose CsA with prednisolone plus ALG for the first 14 days in controlled NHBDs. Outcomes for early function and graft survival to 60 months were significantly better in the low-dose CsA group. Asano et al [117] performed a similar study with these two experimental groups and a further one using low-dose CsA, prednisolone, and azathioprine, and found that this third group had even better outcomes than the ALG group. Using quadruple therapy of low-dose CsA, prednisolone, azathioprine, and ALG [4], better results have been obtained for NHBD kidneys than with other regimens involving normal doses of CsA.

The monoclonal antibody OKT3 has been used for induction therapy, but it has a broad side-effect profile. In one study, 6 of 17 uncontrolled NHBD recipients treated with OKT3 developed neurologic complications, half of who required temporary mechanical ventilation for respiratory support [118].

Mycophenolate mofetil (MMF) and rapamycin are two newer immunosuppressants that are nonnephrotoxic, with potential to replace calcineurin inhibitors in the longer term. The authors' unit now uses low-dose tacrolimus for NHBDs, plus MMF and prednisolone. Daclizumab, a humanized monoclonal anti-IL2 antibody, is a drug of emerging potential. In phase III clinical trials, it has been shown to reduce the incidence of acute rejection at 6 and 12 months after primary cadaveric renal transplantation, without any deleterious effects on renal function or survival [119]. A further study has demonstrated reduced rates of acute rejection in patients with delayed graft function treated with daclizumab [120]. No data have yet been published for the use of interleukin-2 (IL-2) receptor antibodies in NHBD kidneys.

Delayed graft function is so high in NHBD, presumably due to warm ischaemia, but data have shown equivalence in terms of graft survival for HBD and NHBD [15, 35, 40]. Thus, the aim of tailoring immunosuppression for NHBD should be to reduce the need for dialysis treatment during this time and avoid the episodes of acute rejection that occur more commonly during DGF.

Technical approaches

In situ cooling reduces renal oxygen consumption and thereby prevents accumulation of the products of anaerobic metabolism. The technique of kidney cooling prior to retrieval should be easy to initiate, provide reliable hypothermia, and be acceptable to relatives of the donor. There are three approaches.

Intravascular cooling. This is the simplest and most popular method and has been described earlier in this paper. Recent developments include the incorporation of secondary control balloons, allowing the surgeon to be certain the main balloons are correctly inflated. There is now a four-lumen catheter that allows continuous pressure monitoring so that flush pressures are maintained at approximately 70 mm Hg; there is evidence that cortical perfusion is most effective at this pressure [121].

Extracorporeal total-body cooling. Here, oxygenated and cooled blood is used to perfuse the whole body by means of extracorporeal circulation equipment [7, 9, 13, 122–124]. Temperature is monitored using an oesophageal monitor, with a target temperature of 15°C to 18°C. The potential advantage is that the NHBD can be maintained on bypass for long periods while awaiting consent. However, it is a complex technique requiring the expertise of a perfusionist. Recently, Valero, Catiana, and Oppenheimer [125] have shown that *normothermic* perfusion with cardiopulmonary bypass results in lower rates of primary nonfunction and lower rates and shorter duration of delayed graft function for NHBD kidneys than those preserved with either intravascular or total body cooling.

Intraperitoneal cooling alone or combined with intravascular cooling. With this technique a chest tube is inserted into the abdomen through a supraumbilical incision [25]; the organs are cooled and emergency laparotomy is performed to extract the kidneys. The method was developed further by Light et al [126]. By using a closed recirculating system for intraperitoneal cooling and subzero temperature fluids (50/50 alcohol and ice water), they were able to reduce the inferior vena cava temperature to 10°C in 60 minutes. After transplantation, their DGF rates were only 50%, compared to other authors who had rates of up to 100% for NHBD kidneys cooled with the intravascular technique [36, 40].

ETHICS AND LEGAL ISSUES

The principal ethical issues concerning NHBD programs are the use of in situ perfusion prior to consent, the diagnosis of death by cardiac rather than brain-stem criteria, and at what time after pronouncement of death the in situ cooling should be started (the "dead donor rule"). Careful thought and constant reassessment needs to be given to ethical and legal guidelines, so donation can be increased while respecting public concerns. There is considerable variation in the laws of different countries with regards transplantation, and specifically NHBD procurement [127–129]. Further, within a country there may be regional differences dependant on the coroner and local ethics committee guidelines. English law does not require consent for prolonged ventilator support or placement of the DBTL catheter, but catheter placement is an invasive procedure and the family members may not wish for this. The counter-argument is that by placing the catheter and preserving kidneys, more families are given the opportunity to donate [13]. For ideal preservation, the kidneys should be perfused as soon as the 10-minute waiting period is over. There is then more time for a careful discussion with relatives and time for them to reach a considered decision. Relatives' consent for the actual donation is always taken, regardless of evidence of the potential donor's wishes antemortem. It has been found that obtaining consent in two stages may result in refusal of consent for nephrectomy after initially gaining consent for cooling [10]. If methods allowing a longer in situ perfusion time were used (e.g., total body cooling), it would be interesting to see if allowing relatives more time to come to a decision would result in increased donation rates. It would certainly reduce the number of lost opportunities because of failure to contact relatives in a short time period.

The "dead donor rule": How long should we wait?

The dead donor rule is pivotal to NHBD organ donation, and states that the donor should not be killed by the act of donation (i.e., the donor must be dead by cardiac criteria at the time of retrieval). There is a period when the patient is dead by cardiac criteria but not by brain-stem criteria [130] and 10 minutes is considered sufficient for the discrepancy to be corrected. There has been considerable debate over the length of time that the patient should be left [23]. The University of Pittsburgh Medical Center originally suggested that a 2-minute period of electrical asystole with absent femoral pulse was sufficient to confirm death by cardiac criteria [131]. The rationale was that by this time, the *process* of irreversible brain death had begun. The advantage of this short time period was that other organs less resistant to ischemia could be harvested along with the kidneys, and kidney WIT was reduced. Further discussion in Maastricht led to the widespread adoption of the 10-minute dead donor rule.

Clearly, these ethical issues are very important and need to evolve along with changes in public opinion. While attempting to increase available organs for chronically ill patients, there is potential for public misunderstanding of NHBD programs; Caplan [132] suggested this could result in a paradoxical decrease in all donations.

CONCLUSION

NHBD kidneys have the potential to expand the donor pool but need to gain wider acceptance amongst transplant personnel, many of whom are unconvinced of their benefits. The principal concerns are that NHBD kidneys do not function as well as those from other sources. While PNF and DGF are higher in the NHBD, there is no evidence that renal function, graft and patient survival, or quality of life are compromised. There are also concerns about ethics and the burdens of cost and time associated with adopting and running NHBD programs. There is firm evidence that these programs maintain donation rates at a time when other sources are failing to adequately contribute to the donor pool. Developments toward ideal preservation conditions are being made, while techniques of viability assessment will help avoid transplantation of kidneys that will never function. Tailoring of immune suppression for NHBDs, with minimization of nephrotoxicity, may further improve results.

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